Vasomotor effects of L- and D-arginine in stenotic atheromatous coronary plaque

D Tousoulis, G J Davies, C Tentolouris, G Goumas, C Stefanadis, P Toutouzas

Abstract

Objective—To examine the effects of exogenous L- and D-arginine on coronary stenosis vasomotion in relation to stenosis morphology.

Design—Intracoronary infusions of normal saline, L- and D-arginine (50 and 150 µmol/min), and glyceryl trinitrate (250 µg bolus) were given in 24 patients with coronary artery disease and stable angina. Coronary stenoses were classified as smooth or complex (irregular borders). The diameter of the coronary stenoses and their adjacent reference segments was measured by computed quantitative angiography.

Results—During L-arginine infusion a larger proportion of complex stenoses than smooth stenoses dilated by ≥ 10% (p < 0.01), and the magnitude of dilatation was greater at the site of complex stenoses (p < 0.05). Irrespective of the type of morphology there was a positive correlation (p < 0.01) between the severity of stenoses and the magnitude of vasodilatation to L-arginine. The response to glyceryl trinitrate was similar in the two groups. No significant change was found in either group in response to D-arginine.

Conclusions—In patients with coronary artery disease, coronary stenoses—particularly those of complex morphology—dilate in response to the administration of L-arginine but not D-arginine. This finding is consistent with partial deficiency of the substrate for nitric oxide synthesis, L-arginine, at the site of complex stenoses.

Table 1 Clinical and angiographic characteristics of the patients

<table>
<thead>
<tr>
<th>Total group</th>
<th>L-A group</th>
<th>D-A group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean (SD))</td>
<td>59 (7)</td>
<td>58 (7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Previous myocardial infarct (&gt; 6 months)</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Smoking</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Family history</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Mean plasma cholesterol (mmol/l) (mean (SEM))</td>
<td>6.53 (0.31)</td>
<td>6.42 (0.44)</td>
</tr>
<tr>
<td>Mean plasma triglyceride (mmol/l) (mean (SEM))</td>
<td>1.85 (0.16)</td>
<td>1.74 (0.19)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One vessel disease</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Two vessel disease</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Three vessel disease</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Number of lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 50% stenosis</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>&lt; 50% stenosis</td>
<td>20</td>
<td>12</td>
</tr>
</tbody>
</table>

Values are numbers of patients unless stated.

L-A, L-arginine; D-A, D-arginine.

Studies in humans have shown that vascular endothelium plays an important role in the regulation of blood flow by releasing endothelium dependent relaxing factors (EDRF). Nitric oxide, a major component of EDRF, is synthesised from the amino acid L-arginine by a family of enzymes through the L-arginine–nitric oxide pathway. The production of nitric oxide may be stimulated by L-arginine administration. It has also been shown that infusion of L-arginine into the brachial artery augments endothelium dependent forearm vasodilatation and reverses the defective endothelium dependent vasodilatation associated with an increased plasma low density lipoprotein concentration or hypercholesterolaemia. L-arginine administration improves the coronary blood flow response to acetylcholine in patients with normal coronary arteries and hypercholesterolaemia, enhancing nitric oxide generation, and inhibits lesion formation after balloon angioplasty. A recent study showed vasodilatation of coronary stenoses with intracoronary L-arginine, and another demonstrated that arginine produced non-stereospecific peripheral vasodilatation.

It has been shown that complex coronary artery stenoses constrict more than smooth stenoses in response to LNMMA (N-monomethyl L-arginine), indicating enhanced basal production of nitric oxide at the site of these stenoses. It is unknown whether this production can be modified by the administration of the substrate for nitric oxide synthase. Thus, in the present study we examined the coronary vasomotor effects of L- and D-arginine in patients with coronary artery disease and correlated the responses with stenosis morphology.

Methods

Patients

We studied 24 patients (18 men, six women; mean (SD) age 59 (7) years) with chronic stable angina, coronary artery disease, and a positive treadmill exercise test result (≥ 0.1 mV ST segment depression) at between 5 and 7 METS using the modified Bruce protocol. The clinical characteristics of these patients are listed in table 1. Patients were excluded from the study if they had diabetes mellitus, recent myocardial infarction (< 6 months). The response to glyceryl trinitrate was similar in the two groups. No significant change was found in either group in response to D-arginine.
L-arginine in coronary artery disease

297

months), left ventricular hypertrophy (on echocardiography), left ventricular dysfunction (left ventricular ejection fraction < 50%), or valvar heart disease. Hypercholesterolaemia was defined as a fasting serum total cholesterol > 200 mg/dl or serum triglyceride > 150 mg/dl. Antianginal drug treatment was stopped 24 hours before the study. The patients were allowed to use sublingual glyceryl trinitrate as necessary, but no study was performed within three hours of its administration.

The protocol was approved by the research ethics committee and each patient gave written informed consent.

PROTOCOL

Following the diagnostic coronary angiogram, an optimal radiographic projection was selected and kept constant for subsequent angiograms. Two ECG leads were monitored continuously throughout the study. The following sequence of intracoronary infusions was applied in 15 patients (11 men, four women): (1) 0.9% saline (2 ml/min) for two minutes; (2) 50 µmol/min of L-arginine for eight minutes; (3) 150 µmol/min of L-arginine for eight minutes; (4) a bolus of glyceryl trinitrate (250 µg in 2 ml of saline). A syringe pump was used for continuous infusions. In nine patients (seven men, two women) with coronary artery disease, the same protocol was performed substituting 50 and 150 µmol/min of D-arginine for L-arginine.

Femoral arterial pressure and heart rate were recorded during the last 30 seconds of each infusion period. Angiography was performed after a hand injection of 6–8 ml non-ionic contrast medium at baseline, immediately after each infusion, and 2–3 minutes after glyceryl trinitrate. Before each angiogram, the catheter was emptied to avoid bolus administration of the infusate.

QUANTITATIVE CORONARY ANGIOGRAPHY

The arterial segments in each frame were analysed in random order using quantitative computed analysis with an automated edge contour detection analysis system (Computerised Angiographic Analysis System (CAAS), version 2V2; Pie Data Medical, Maastricht, The Netherlands). End diastolic frames from each arteriogram were selected for analysis. The angiographic catheter was used as a scaling device and this, together with the pin cushion distortion correction, allowed the diameters to be recorded as absolute values (expressed in mm). Stenoses were morphologically classified as smooth (concentric or eccentric) or “complex” by two blinded independent observers on the basis of visual inspection of arteriograms recorded in two orthogonal projections. This classification of stenosis was compared with that obtained by computed symmetry analysis (CAAS symmetry index).% of the same coronary lesions. Concentric stenoses were defined as those producing symmetrical narrowing, with smooth borders or only very mild irregularity (symmetry index > 0.5–1) that looked similar in orthogonal projections. Eccentric stenoses were defined as asymmetrical narrowing with smooth borders and a broad neck (symmetry index 0.0–< 0.5). Complex stenoses were defined as asymmetric narrowing with irregular borders and/or overhanging edges (type II of Ambrose can colleagues) or with an “abrupt proximal face” or a “rough” or “sawtooth” component.

Quantitative analysis of coronary arteriograms was carried out by two independent observers, who blindly reanalysed the films at a remote time for reproducibility of the method. No significant intraobserver or interobserver variability was found (analysis of variance F = 0.37, p = 0.8).

STATISTICAL ANALYSIS

Data are expressed as mean (SEM). Analysis of variance (ANOVA) and the Scheffé F test for repeated measures were used to compare serial changes in heart rate and blood pressure and in diameter of coronary stenoses. To test for differences in response of smooth and complex stenoses to L- and D-arginine and nitrates, a two way ANOVA for repeated measures was applied. Associations between responses to L-arginine and stenosis length, severity, and eccentricity ratio were assessed by performing linear regression analysis and calculating a correlation coefficient. Student’s t test was used to compare paired and unpaired data between groups, and the responses to glyceryl trinitrate and L- and D-arginine. A probability value of p < 0.05 (two tailed) was considered significant.

Results

The clinical and angiographic characteristics of the patients are listed in table 1. Systolic aortic pressure and heart rate remained unchanged during intracoronary administration of L-arginine (blood pressure 143.2 (5.6) v 146.2 (5.9) mm Hg; heart rate 70.1 (1.8) v 72.5 (1.8) beats/min during baseline and L-arginine, respectively), and also during intracoronary administration of D-arginine (blood pressure 146 (8) v 145 (9) mm Hg; heart rate 67.3 (2.6) v 68.1 (1.8) beats/min during baseline and D-arginine, respectively).

STENOSIS MORPHOLOGY AND RESPONSE TO L-ARGININE AND GLYCERYL TRINITRATE

Twenty two of the 26 coronary stenoses (12 smooth, 10 complicated) observed in these 15 patients were suitable for quantitative analysis, and the results below refer to these stenoses. The severity of coronary stenoses for the whole group ranged from 22.2–86% luminal diameter reduction (mean 48.2 (3)%). There were 10 stenoses of 50%: four smooth, six complex.

During L-arginine infusion a larger proportion of complex stenoses than smooth stenoses dilated by 10% (50% v 21%, p < 0.01). The magnitude of dilatation was greater (p < 0.05) at the site of complex stenoses than at the site of smooth stenoses, but was similar in their reference segments (table 2; fig 1). Irrespective of the type of morphology, there
was a positive correlation (p < 0.01) between the severity of stenoses and the magnitude of the vasomotor response to L-arginine (fig 2). A similar proportion of smooth and complex stenoses showed ≥ 10% dilatation with glyceryl trinitrate (67% v 80%, NS), and the magnitude of the response was similar in the two groups (table 2). In response to 150 µmol/min
L-arginine, there was no difference in the magnitude of dilatation of coronary stenoses between smokers and non-smokers (11.2 (2.2)% vs 10.2 (5.2)%, respectively; NS), between hypercholesterolaemic and non-hypercholesterolaemic patients (10.2 (2.8)% vs 11.9 (3.0)%; NS), or between hypertensive and non-hypertensive patients (10.7 (3.5)% vs 11.2 (2.5)%; NS).

**Table 3** Reactivity of coronary stenoses and their reference segments to intracoronary administration of D-arginine (D-A) and nitrates

<table>
<thead>
<tr>
<th>Minimum lumen diameter (mm)</th>
<th>Stenosis</th>
<th>Reference segments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>DA-50</td>
</tr>
<tr>
<td>Smooth (n=8)</td>
<td>1.64 (0.11)</td>
<td>1.66 (0.11)</td>
</tr>
<tr>
<td>Complex (n=10)</td>
<td>1.51 (0.14)</td>
<td>1.52 (0.14)</td>
</tr>
</tbody>
</table>

Values are mean (SEM).

DA-50, 50 µmol D-arginine/min; DA-150, 150 µmol D-arginine/min.

**Discussion**

In this study we investigated the effects of L- and D-arginine and the endothelium independent vasodilator glyceryl trinitrate in patients with coronary artery disease and stable angina. Complex stenoses were more likely to dilate in response to L-arginine than smooth stenoses, and they dilated to a greater degree. There was no significant response of either type of stenosis to D-arginine. The response to glyceryl trinitrate was independent of stenosis morphology.

**PLAQUE MORPHOLOGY AND NITRIC OXIDE ACTIVITY**

Clinical studies have suggested that stenoses with a complex morphology rapidly progress to total or subtotal occlusion and are often the substrate for acute coronary syndromes. These stenoses are not only morphologically complex but also have more complex histology and contain a large variety of cell types including abundant macrophages. They are also more complex functionally, as exemplified by an enhanced vasoconstrictor response to serotonin and other vasoconstrictor stimuli. Results of our previous studies showing enhanced constriction in response to LNMMA, suggestive of increased nitric oxide synthase activity, indicate that the pathologically enhanced vasoconstrictor responsiveness is also expressed in the endogenous vasodilator mechanisms within these stenoses. This finding is consistent with the observation of inducible nitric oxide synthase immunoreactivity within complex atherosclerotic coronary artery stenoses. The cellular localisation of this nitric oxide synthase is unclear but it could possibly be in macrophages and smooth muscle cells, and it has been found in the endothelium of the new microvessels in the wall of the artery.
around the atheromatous plaque. Such neo-
vascularisation of atheromatous plaques is well
documented.

The resting tone appears to be similar for both smooth and complex stenoses, as evi-
denced by the similar magnitude of dilatation in
response to nitrate administration. However,
our results provide further evidence that com-
plex plaques are active structures. There may be
a link between enhanced inducible nitric
oxide activity (produced by macrophages) and
plaque instability. Macrophages and the T
lymphocytes may produce cytokines that in-
duce nitric oxide synthase. Furthermore,
metalloproteinases produced in macrophages
in vulnerable regions of complex atheroscle-
rotic plaques may weaken the fibrous cap of the
plaque, leading to rupture and thrombosis. There is also growing evidence that macro-
phages may be involved in smooth muscle cell
death by apoptosis which occurs in atheroma-
tous plaques and that they initiate or enhance
the degradation of collagen.

PLAQUE MORPHOLOGY AND RESPONSE TO L- AND
D-ARGININE

L-arginine, whether given intravenously or
intrathecally, can reduce vascular tone. The
mechanism by which it exerts its vasodila-
tor effects is controversial, but the L-arginine–NO synthase nitric oxide pathway
appears to be particularly important. The L
isomer of arginine is a substrate for the
endothelial cell and for both inducible (in
macrophages and foam cells) and smooth
muscle cell isoforms of the enzyme nitric oxide
synthase. These enzymes convert L-arginine
to citrulline and nitric oxide. The D isomer of
arginine is not a substrate for nitric oxide syn-
thase.

It has been suggested that diseased arteries
may be relatively deficient in the substrate
L-arginine. Apart from limiting nitric oxide
production, substrate deficiency could lead to
the generation of superoxide by both inducible and endothelial nitric oxide syn-
thase. The results of our study are consistent
with a relative deficiency of L-arginine at the
site of stenoses in diseased coronary arteries,
particularly within stenoses with complex mor-
phology. They are a further indication that
complex morphology is a marker of increased
functional activity and they are consistent with enhanced nitric oxide activity. This could
represent a natural compensatory mechanism
to counteract the predisposition to constriction
generated by atherosclerotic disease. A recent
study showed that parenteral arginine pro-
duced non-stereospecific peripheral vasodilata-
tion and improved endothelium dependent
vasodilatation in patients with stable coronary
artery disease by stimulation of insulin depend-
cent nitric oxide release or by non-enzymatic
nitric oxide generation. Other studies also
showed a non-stereospecific arteriolar and
venous dilatation accompanied by hypotension
in normal subjects at high parenteral concen-
trations of both L- and D-arginine. In contrast,
intravenous L-arginine but not D-arginine
improved forearm dilatation in hypercholes-
terolaemic subjects in response to metha-
choline. Furthermore, in coronary arteries,
intravenous L-arginine but not D-arginine
improved the acetylcholine responses in
hypercholesterolaemic and atherosclerotic patients. Panza and colleagues showed that
availability of the substrate for production
of nitric oxide is a rate limiting step for endothelial dependent vascular relaxation in
normal healthy subjects but not in hypertensive
patients. Oral arginine has also been shown to
improve brachial artery flow mediated dilata-
tion in hypercholesterolaemic patients but not
in normal individuals. We found that intra-
arterial L-arginine significantly dilated
atherosclerotic arteries and stenoses in patients
with stable angina. Although there was a posi-
tive correlation between the severity of stenoses
and the magnitude of the vasomotor response
to L-arginine, it was weak ($r = 0.56$).

The high intracellular concentrations of L-arginine found in experimental studies suggest that a deficiency of this substrate may not be responsible for reduced availability of nitric oxide. Thus other mechanisms should be postulated to explain the beneficial effects of L-arginine. These include reversal of inhibitory effects of L-glutamine on L-arginine; counter-
action of inhibitory effects of naturally occurring asymmetrical dimethyl arginine (ADMA); antioxidant effects; insulin release; and non-
enzymatic generation of nitric oxide by
L-arginine. The effects of dietary arginine
supplementation on endothelium-dependent coronary vasodilatation and responses to acetylcho-
line. 

CONCLUSIONS

In patients with coronary artery disease,
complex coronary stenoses dilate more than
smooth stenoses after L-arginine administra-
tion, but neither respond to D-arginine. This is consistent with partial deficiency of the sub-
strate for nitric oxide synthesis at the site of
atheromatous stenoses, particularly when they
are of complex morphology.

1 Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology
2 Vanhoutte PM. The endothelium. Modulator of vascular
3 Luecher TF, Richard V, Tschudi M, et al. Endothelial con-
trol of vascular tone in large and small coronary arteries. J
4 Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial
cells synthesize nitric oxide from L-arginine. Nature 1988;
5 Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release
accounts for the biological activity of endothelium-derived
6 Rees DD, Palmer RMJ, Hodson HE, et al. A specific inhibi-
tor of nitric oxide formation from L-arginine attenuates
endothelium-dependent relaxation. Br J Pharmacol 1989;
7 Creager MA, Gallagher SM, Giered XJ, et al. L-Arginine
improves endothelium-dependent vasodilation in hyperc-
8 Inamura T, Hirooka Y, Masaki H, et al. Effects of L-arginine
on forearm vessels and responses to acetylcho-
9 Jeremy JW, McCarron H, Sullivan D. Effects of dietary
L-arginine on atherosclerotic and endothelium-dependent
vasodilation in the hypercholesterolaemic rabbit: response
according to treatment duration, anatomic site, and sex.
10 Drexler H, Zeiher AM, Meinzer K, et al. Correction of
endothelial dysfunction in coronary microcirculation of
hypercholesterolaemic patients by L-arginine. Lancet 1991;
338:1546–50.
supplementation on endothelium-dependent coronary va-
sodilation in patients with angina pectoris and normal cor-
L-arginine in coronary artery disease


7th European Forum on Quality Improvement in Health Care
21–23 March 2002
Edinburgh, Scotland

We are delighted to announce this forthcoming conference in Edinburgh. Authors are invited to submit papers (call for papers closes on Friday 5 October 2001) and delegate enquiries are welcome.

The themes of the Forum are:
- Leadership, culture change, and management
- Achieving radical improvement by redesigning care
- Health policy for lasting improvement in health care systems
- Patient safety
- Measurement for improvement, learning, and accountability
- Partnership with patients
- Professional quality: the foundation for improvement
- Continuous improvement in education and training
- People and improvement.

Presented to you by the BMJ Publishing Group (London, UK) and Institute for Healthcare Improvement (Boston, USA). For more information contact: quality@bma.org.uk or look at the website www.quality.bmj.org. Tel: +44 (0)20 7383 6409; fax: +44 (0)20 7373 6869.